

Mating Behavior in Three Species of the *Drosophila hypocausta* Subgroup

NOBUHIKO ASADA¹, KYOKO FUJIWARA², HIROSHI IKEDA^{3,*}
and FUYUO HIHARA³

¹Biological Laboratory, Faculty of Science, Okayama University of Science,
Okayama 700, Japan, ²Department of Biology, Faculty of Science and

³Biological Institute, Faculty of General Education,
Ehime University, Matsuyama 790, Japan

ABSTRACT—Courtship behavior was evaluated to investigate the degree of sexual isolation in three related species, *Drosophila hypocausta*, *D. neohypocausta* and *D. siamana*. Patterns of male courtship behavior and the nature of courtship sounds using an oscilloscope were compared among these species. *D. siamana* was ethologically isolated incompletely from *D. hypocausta*; *D. siamana* females were highly receptive to *D. hypocausta* males, giving the average rate of insemination of 67.1%, although the reciprocal crosses gave only 13.4%. The nature of the courtship behavior and courtship sounds emitted by males showed species-specific patterns. Results of crossability tests and behavioral analyses suggested that *D. siamana* is a good species of the *D. hypocausta* subgroup of the *D. immigrans* species group.

INTRODUCTION

The *Drosophila hypocausta* subgroup consists of seven species including *D. hypocausta* and *D. neohypocausta*. Collecting expeditions of *Drosophila* flies were carried out in 1979 and 1981 in southeast Asia and many specimens were collected in Malaysia and Thailand. *D. hypocausta* inhabits wider areas in southeast Asia including Thailand, Malaysia, Philippines and Papua New Guinea. *D. neohypocausta* is considered to be an endemic species of Taiwan, and *D. siamana* inhabits in Thailand and Malaysia. According to the morphological analysis, *D. siamana* closely resembles *D. hypocausta* in general features, but differs from the latter in the shape of aedeagus and in having larger C3 fringe and two prominent bristles on the metatarsus of the hind legs [1].

Courtship sounds, particularly male sounds, are important for species recognition in *Drosophila* [2-4] as well as in other animals [5, 6]. Intrapulse

frequency and the pulse repetition rate, or interpulse interval (ipi), of courtship sounds emitted by males are thought to be important factors of species discrimination for the female in acceptance of copulation [7, 8]. These findings suggest that interspecific differentiation of courtship sounds emitted by the male might act as an incipient sexual isolation mechanism during the course of speciation.

In this article, the degree of reproductive isolation, especially sexual isolation, is analyzed and the nature of male courtship behaviors and sounds among three species is also presented.

MATERIALS AND METHODS

Flies

The following species and strains were used: *D. hypocausta*, R164; collected at Palawan island of Philippines in 1979, W103; collected at Singapore in 1979, *D. neohypocausta*, I-Lan; collected at Chung-tou of Taiwan in 1979, *D. siamana*, Y110 and Y115; collected at Penang island of Malaysia in 1979, Z17 and Z28; collected at Nakhon Nayok

Accepted February 17, 1992

Received July 7, 1991

¹ To whom all correspondence should be addressed.

* Deceased on April 2nd, 1987

of Thailand in 1979. R164, I-Lan and Y110 strains were used for behavioral analysis. All strains were wild caught iso-female origin. Flies were reared on standard cornmeal-yeast medium at 25°C under the artificial light and dark cycle (LD=12:12). Evaluation of mating behavior and the recording of courtship sounds were performed during the light period because these species were sexually active in the morning (Asada, unpublished data).

Cross experiments

Mating propensity was calculated as the number of matings divided by the total number of females tested. Five females and eight males were put together in a glass vial (30×110 mm) for two days. To determine whether copulation had occurred or not, females were dissected and examined for the presence of sperm in the females' internal reproductive organs. Approximately 100 females were tested.

Evaluation of mating behavior

In order to analyze male courtship behavior and repelling actions of females, two pairs of flies were put in a mating chamber (30 mm in diameter), and mating behaviors of both sexes were evaluated. The terminology of behaviors was described following Spieth [9]. To evaluate the duration of copulation, approximately 30 pairs were placed together in a glass vial.

Procedures for detecting the sound were same as

those of Ikeda *et al.* [8]. The main equipment included an oscilloscope, Nihon Kodens VC-7A; an amplifier, Nihon Kodens AVB-9 and AVH-9; a microphone, Nihon Kodens MSC-It; a camera, Nihon Kodens PC-2B; and a data recorder, Sony DFR-3415. A recording cell (3×30 mm diameter) was equipped with a microphone diaphragm, and the cell was placed inside a soundproof box. Un-anesthetized virgin flies, two females and one male, were put into the cell. Inter pulse interval (ipi) and number of cycles per pulse, and number of pulses per burst were counted in 25 samples. Male courtship sounds of *D. hypocausta* and *D. siamana* were recorded in 25 replicates for each five copulated pairs.

RESULTS

Crossability and productivity among three species

Proportions of successful matings among three species are shown in Table 1. The average rates of females inseminated in intraspecific crosses were 84.9, 84.8 and 90.5% on average, respectively. In interspecific crosses, the rate of insemination varied from zero to 94%. No interspecific cross was found between *D. neohypocausta* and the other two species, showing complete ethological isolation between them. *D. siamana* females were highly receptive to *D. hypocausta* males, giving the average rate of insemination of 67.1%, whereas

TABLE 1. Percent of successful matings in intra- and interspecific crosses among three species of the *D. hypocausta* subgroup

| Female \ Male | <i>D. hypocausta</i> | | | <i>D. neohypocausta</i> | | <i>D. siamana</i> | | | | |
|----------------------|----------------------|--------|---------|-------------------------|-------------|-------------------|--------|--------|---------|--------------|
| | R164 | W103 | Average | I-Lan | Y110 | Y115 | Z17 | Z28 | Average | |
| <i>hypocausta</i> | R164 | [98.2] | 75.0 | 87.1 (210) | 0.0 (50) | 14.0 | 12.0 | 22.0 | 0.0 | 12.0 (400) |
| | W103 | 80.0 | [85.0] | 82.5 (200) | 0.0 (50) | 24.0 | 14.0 | 17.7 | 4.0 | 14.8 (400) |
| | Total | 89.5 | 80.0 | [84.9](410) | 0.0 (100) | 19.0 | 13.0 | 19.5 | 2.0 | 13.4 (800) |
| <i>neohypocausta</i> | I-Lan | 0.0 | 0.0 | 0.0 (100) | [84.8](125) | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 (200) |
| <i>siamana</i> | Y110 | 94.0 | 69.0 | 81.5 (200) | 0.0 (50) | [97.9] | 98.0 | 100.0 | 97.0 | 98.2 (440) |
| | Y115 | 71.0 | 54.0 | 62.5 (200) | 0.0 (50) | 69.0 | [77.2] | 65.0 | 77.0 | 72.6 (445) |
| | Z17 | 55.0 | 57.0 | 56.0 (200) | 0.0 (50) | 98.0 | 98.3 | [97.4] | 98.0 | 97.9 (470) |
| | Z28 | 76.0 | 61.0 | 68.5 (200) | 0.0 (50) | 93.6 | 98.3 | 91.0 | [89.7] | 92.9 (505) |
| | Total | 74.0 | 60.3 | 67.1 (800) | 0.0 (200) | 90.4 | 91.9 | 89.5 | 90.3 | [90.5](1860) |

Number in parenthesis: number of females dissected.

the reciprocal crosses gave the lower rate of insemination, 13.4% on average.

Mating experiments were also carried out in complete dark for 48 hr using *D. hypocausta* and *D. siamana*. These two species were considered to require light to mate, because none of the 100 females tested was found to be inseminated in each intraspecific crosses. The average duration of copulation in min of *D. hypocausta*, *D. neohypocausta* and *D. siamana* was 8.8 ± 0.5 , 9.7 ± 0.33 and 14.9 ± 0.70 , respectively. No significant difference of the average duration of copulation was found between *D. hypocausta* and *D. neohypocausta*, although that of *D. siamana* was significantly longer than those of the others at 0.1% level by t-test.

D. hypocausta females produced fertile females and sterile males when crossed to *D. siamana* males. The reciprocal crosses, however, produced no viable F₁ flies, although a few of the 1st instar larvae was found in the cultures.

Mating behavior

Schematic representations of mating behaviors are shown in Figures 1 and 2. Male courtship sounds of *D. hypocausta* and *D. siamana* were emitted together with behaviors shown by bold-boxes in Figure 2.

D. hypocausta: The male sighted a moving female approaches to the female, then repeatedly taps (Ta) the female's body, and frequently flicks (F) both wings approximately at an angle of 80° and vibrates (V) them at the same time (A-1). The pulse sound emitted by flicking-vibration is designated as the FVp sound. Then the male approaches a female much more closely behind, places his head under female's wings, extends one wing to approximately at an angle of 20–40°, vibrates both wings (A-2). The sine sound (humming sound, [10]) produced during these behaviors is referred to the LVs. Finally the male attempts to

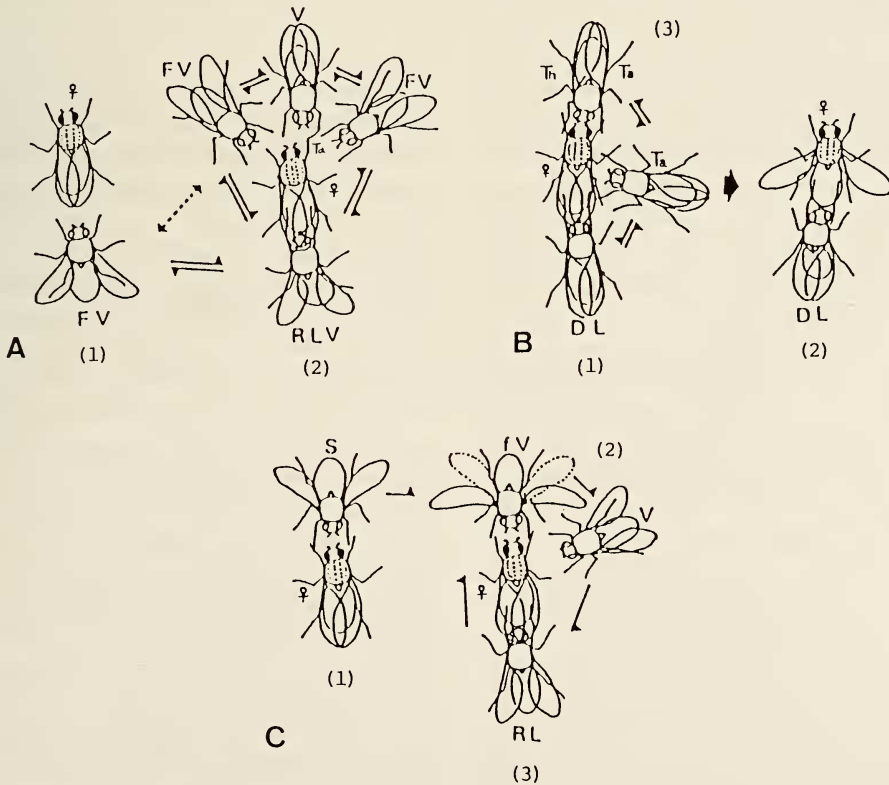


FIG. 1. Courtship behaviors of three species belonging to the *D. hypocausta* subgroup.

A: *D. hypocausta*, B: *D. neohypocausta*, C: *D. siamana*. F: flicking, f: flapping, V: vibration, S: shivering, D: drumming, R: rubbing, Ta: tapping, Th: thrusting, L: licking.

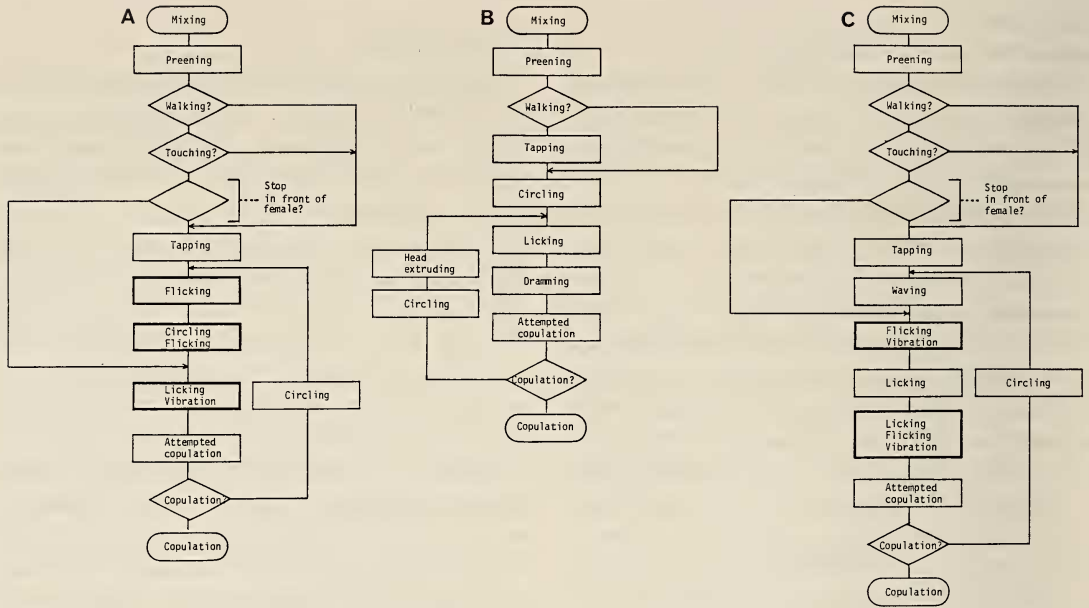


FIG. 2. A flow chart of courtship behaviors of males in three species.

A: *D. hypocausta*, B: *D. neohypocausta*, C: *D. siamana*. Courtship songs are emitted in movements shown by bold-boxes.

mount. During wing vibration, the male continuously licks or tries to lick the female's genitalia, and rubs (R) the anterior lateral surface of the female's abdomen with his forelegs. Thus, the female may receive three, at least, different kinds of stimuli at the same time from the courting male.
D. neohypocausta: The male sighted a female approaches, then repeatedly taps the female's body and moves behind her. He places his head under female wings, then drums (D) the middle dorsal surface of the female's abdomen with his forelegs persistently for a few sec. At the same time, the male continuously licks (L) or tries to lick the female's genitalia (B-1). The receptive female gradually spreads both wings to approximately at an angle of 100° at its maximum, then finally permits the male to mount (B-2). When the female is unreceptive, the male moves in front of the female with a crab-like motion. While moving, the male always tries to tap the female's body. The male positioning himself in front of the female frequently thrusts (Th) at the female with his head (B-3). Thereafter, the male moves back behind the female. The male never displays wing motion during courtship.

D. siamana: The male sighted the female places himself directly in front of the female's head. He extends his hind legs so that the body is inclined to be lower side. The male raises and spreads both wings to approximately at an angle of 90° and slightly shivers for several sec. in the inclined posture; the pulse sound emitted is hardly detectable (C-1). Thereafter the male quickly flaps (f) both wings three to four times to approximately at an angle of 180° at its maximum, emitting the pulse sound, fVp. Just after several wing-flappings, the male spreads one wing to approximately at an angle of 20-40° and vibrates it for a moment, producing the pulse sound, Vp, which is followed by the irregular sine sound, Vs (C-2). Then the male quickly moves behind the female in the crab-like manner, and displays behaviors emitting either the Vs sound or the fVp+Vp sounds. The male tries to lick the female's genitalia and to rub the lateral surface of the female's abdomen with his forelegs in the head-under-wings posture, however, these are less frequent compared with the male of *D. hypocausta* (C-3).

Figure 2 shows the flow chart of the typical case of the courtship behaviors of three species de-

scribed above. Those of *D. hypocausta* and *D. siamana* are very similar each other. The flicking and vibration sounds, however, are quite different as shown in later. The flow chart of the courtship behaviors of *D. neohypocausta* is clearly different from the other two species; it lacks flicking and vibration of the wings and involves drumming behavior which was not observed in the other species.

The nature of male courtship sounds

In order to analyze the nature of male courtship sounds, an oscillogram of both *D. hypocausta* and *D. siamana* were examined. *D. neohypocausta* could not be used because the male of this species showed no wing movement at all.

D. hypocausta: The male emitted four kinds of courtship sounds, FVs, hVs, FVp and LVs, which were clearly distinguished from each other and from those of *D. siamana* males by oscilloscope patterns (Fig. 3A). The FVs sound was a sine sound lasting less than 50 msec. The hVs sound was a sine sound with a low amplitude. The FVp sound was a burst consisting of a train of pulses comprising 2 to 4 cycles. The average ipi was 7.8 ± 0.2 msec and the average of number per burst was 15.2 ± 1.5 . This sound showed to be harmonic.

The LVs sound was a burst consisting of a train of sine sounds each of which (defined as a unit) lasted 321.07 msec on average, ranging between 227.34 and 454.26 msec. The average number of the units per burst was 19.4, the total length of a burst being approximately 6 sec. The frequency was changed within a unit of the sine sound; it started with a minimum frequency, reached a maximum and then returned to the minimum.

D. siamana: The male emitted three kinds of courtship sounds, fVp, Vp and Vs (Fig. 3B). The fVp sound was followed by the Vp sound with an average interval of 68.3 msec. The Vs sound always followed immediately after the Vp. Thus, a set of three sounds was produced by serial courtship behaviors including wing flapping, spreading and vibration, however it was not able to distinguish behaviors between emitting Vp and Vs. The sine sound with a low amplitude was detected when the male extended a single wing and vibrated both wings in the head-under-wings posture behind the female. According to the oscilloscopic pattern, the nature of this sound was essentially the same as the Vs sound, however the former lasted longer than the latter. The fVp sound consisted of 2 to 4 pulses (one pulse being emitted single wing flapping) each of 4 to 5 cycles, with

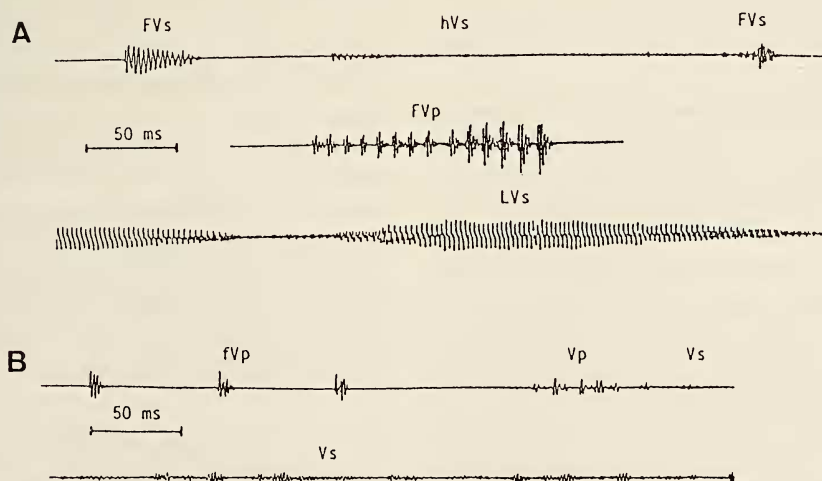


FIG. 3. Oscilloscope patterns of courtship sounds emitted by courting males.

A: *D. hypocausta*, B: *D. siamana*. FVs: sine sound emitted by flicking-vibration, hVs: sine sound emitted by flicking-vibration, FVp: pulse sound emitted by flicking-vibration, LVs: sine sound emitted by flicking-vibration, fVp: pulse sound emitted by flapping-vibration, Vp: pulse sound emitted by flapping, Vs: sine sound emitted by vibration. For details, see the text.

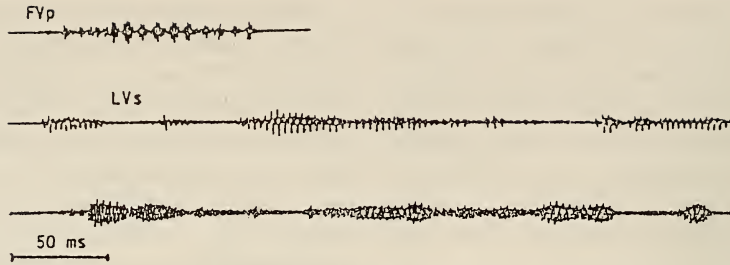


FIG. 4. Oscilloscope patterns of courtship sounds of the F₁ male obtained by crossing between *D. hypocausta* and *D. siamana*. For abbreviations, see legend to Fig. 3.

TABLE 2. Courtship behaviors either of *D. hypocausta*- or of *D. siamana*-type in males of the backcross generation (BC1)

| [(<i>hypo</i> ♀ × <i>sia</i> ♂)F ₁ ♀ × <i>hypo</i> ♂] → BC1 ♂ | | | |
|---|----------------------|----|-------|
| <i>hypocausta</i> -type | <i>siamana</i> -type | no | Total |
| 19 | 0 | 7 | 26 |
| [(<i>hypo</i> ♀ × <i>sia</i> ♂)F ₁ ♀ × <i>sia</i> ♂] → BC1 ♂ | | | |
| <i>hypocausta</i> -type | <i>siamana</i> -type | no | Total |
| 19 | 14 | 12 | 45 |

hypo: *D. hypocausta*, *sia*: *D. siamana*, no: no courtship display.

average ipi of 68.3 msec. The Vp sound was a burst consisting of a train of pulses with the average ipi of 12.3 msec and average number of pulses per burst of 7.2.

The nature of courtship sound in F₁ and backcrossed hybrids

The genetic basis of the courtship behaviors was examined using F₁ and back-crossed hybrid males (sterile) occurred between *D. hypocausta* and *D. siamana*. Results are shown in Figure 4. The courtship behavior of the F₁ hybrid males originated from crosses between *D. hypocausta* females and *D. siamana* males was similar to that of *D. hypocausta* males except lacking wing display in front of females, and the courting male emitted two kinds of courtship sounds, FVp and LVs. LVs sound of hybrid males could be clearly distinguished from that of *D. hypocausta* males by oscilloscope patterns showing an irregular pattern. No sound resembling to that emitted by *D. siamana* males could be detected in F₁ hybrid males.

Back-crosses (BC1) males were emerged from crosses in F₁ females originated from crosses be-

tween *D. hypocausta* females and *D. siamana* males and *D. hypocausta* (or *D. siamana*) males. Results of the BC1 male courtship behavior are summarized in Table 2. *D. hypocausta*-type behavior involves a series of licking-rubbing-vibration, and that of *D. siamana*-type involves flapping. When BC1 males were derived from the crosses between hybrid females and *D. hypocausta* males, only *D. hypocausta*-type behavior was observed. BC1 males derived from the back crosses of F₁ females to *D. siamana* males segregated into statistically equal number of flies having *D. hypocausta*-type behaviors or *D. siamana*-type behavior. Thus, *D. siamana*-type behavior seemed to be controlled by an autosomal recessive gene(s) allelic to dominant gene(s) of *D. hypocausta*.

DISCUSSION

The male of *D. hypocausta* and *D. siamana* are readily distinguishable from each other by the degree of coloration of the body; the aged male of *D. hypocausta* is characterized by a black abdomen, thorax and legs, while the male of *D. siama-*

na has a dark brown abdomen, thorax and brown legs [1]. It is hard to distinguish females of the two species from each other on the basis of external morphology. *D. hypocausta* females are much more discriminatory in acceptance of males than *D. siamana* females. This may be closely associated with facts that *D. hypocausta* males emit simultaneously at least three kinds of stimuli which are released through licking, rubbing and wing vibration behind the female, whereas the male of *D. siamana* less frequently displays these behaviors. LVs sound emitted by males of *D. hypocausta* is species-specific song and is not observed in the other *Drosophila* species.

For three species, it may be true that visual stimuli are important to find and/or to discriminate the partner, showing that these species are completely dependent on light for copulation; no copulation occurred for 48 hr in the dark for *D. hypocausta*, *D. siamana* and possibly for *D. neohypocausta*. The importance of visual stimuli is suggested by another fact that males of the three species moved around the female in a crab-like behavior, always facing the female, during courtship.

Auditory stimuli may not be included in the SMRS [11] of *D. neohypocausta*, since the male never showed wing displays during courtship. The male of this species persistently tries to contact physically with the female through tapping, thrusting and drumming motions when he is courting. Essential stimuli responsible for mating success, thus, may be chemical as well as visual [12].

It is of interest that there are significant differences in courtship behaviors between *D. hypocausta* and *D. siamana* which are thought to be closely related species on the basis of morphology and the interspecific hybridization test. Usually, differences both in the behavioral pattern and in the nature of the stimulus are not qualitative but quantitative between closely related species. For example, Spieth [9] found differences in only three elements out of 19 visible courtship behaviors tested between *D. melanogaster* and *D. simulans*. Ewing and Bennet-Clark [2] could not reveal the difference in the oscilloscopic pattern except for the ipi between these species. The ipi is thought to be one of the most important characters for the

discrimination of species in related species.

Results obtained from hybridization tests suggested that *D. hypocausta*-type behaviors such as emitting FVp and LVs sounds may be determined by autosomal dominant genes. *D. siamana*-type behaviors were found only in the back-crossed generation, suggesting that genes are autosomal recessive. Thus, it is likely that behavioral differences including courtship sounds between these species are based on genetic differences. As mentioned above, *D. siamana* was reconfirmed as species belonging to the *D. hypocausta* subgroup through genetic and behavioral analyses. However, the evolutionary process to differentiate the genetic system controlling courtship behaviors between species may be a subject for futures studies.

ACKNOWLEDGMENTS

This work was mainly supported by the funds of the Overseas Scientific Expedition in 1971 (No. 7114), 1979 (No. 404149) and 1980 (504344), and Grant-in-Aid (No. 548001) from the Ministry of Education, Science and Culture of Japan.

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